MIDDLE BOULDER CAÑON

MYCOLOGIA

VOL. XXII JANUARY–FEBRUARY, 1930 NO. 1

A MYCOLOGICAL FORAY THROUGH THE MOUNTAINS OF COLORADO, WYOMING AND SOUTH DAKOTA

FRED J. SEAVER AND PAUL F. SHOPE

(WITH PLATES 1–7)

During the summer of 1929, after an extended correspondence, between the senior author, of The New York Botanical Garden, and the junior author, of the University of Colorado, plans were finally consummated for a joint expedition into the mountains of Colorado and adjacent states in search of fungi. Boulder was made the base of these operations and the authors joined forces there on the 22d of July.

On the following day a temporary sub-base was established in Middle Boulder Cañon at an elevation of 10,000 feet, in the deserted mining settlement of the U. S. Gold Corporation. Selecting one of the least dilapidated cabins of the settlement, the party consisting of the writers and Mr. M. O. Jung, proceeded to make themselves comfortable (PLATE 1). The only furnishings of this shack consisted of a small stove and a pile of straw. Both were appreciated since at this elevation the camp was just below the regions of perpetual snow which still hung in the gulches above, and the temperature at night fell to, or approached, the freezing point. During the daytime, however, it was very comfortable and the weather was favorable for collecting, an unusual condition since daily rains frequently occur at this altitude.

The predominating trees in this region consisted of spruces

[MYCOLOGIA for November–December (**21**: 301–348) was issued November 1, 1929]

(*Picea Engelmanni*), limber pine (*Pinus flexilis*) and firs (*Abies lasiocarpa*) and, since the distribution of fungi is governed more by the substratum than by the temperature and altitude, an especial effort was made to collect the forms which occurred on and under these trees. Two of the commonest cup-fungi in the region were *Dasyscypha Agassizii* and *Dasyscypha arida*, both rather conspicuous by reason of their brilliant color. One of the most interesting cup-fungi found here was apparently *Paxina nigrella*, previously known only from a single collection obtained by Dr. L. O. Overholts at Tolland. This was found commonly around the edges of the melting snow drifts and much material was added to our formerly scant collection. The great profusion of spring flowers, especially dog-tooth violets growing in close proximity to the melting snows in July presented, not only a charming spectacle but was of real interest to one not familiar with these conditions. The highest elevation visited here was about 12,000 feet.

After returning to Boulder and caring for the material collected, the base of operations was transferred to the University of Colorado Summer Camp, located at an elevation of 9,600 feet, where the coniferous forests were supplemented by dense growths of aspens (*Populus tremuloides*) (PLATE 3). This camp was in charge of Dr. W. E. McCourt who kindly extended to the writers all the privileges of the camp. Much interest was shown by the science students in the many boletes and agarics which abounded at this time and the writers were frequently questioned as to their edibility. Several pleasant evenings were spent around the large camp-fire in conferences presided over by Dr. McCourt and his corps of able assistants. Here the partially buried cup-fungus, *Sepultaria aurantia*, previously known in this country only from the type locality in Nebraska, was collected in abundance. The color of the interior of the apothecia is pale yellow, becoming decidedly orange as they mature and begin to dry out.

This species was originally described by Dr. F. E. Clements and was listed in The North American Cup-fungi with some misgivings since at that time we had no material and were compelled to rely on the record alone. The material obtained is

MIDDLE BOULDER CAÑON

therefore a most valuable addition to our collection. As intimated in The North American Cup-fungi, this species is probably identical with the earlier European species, *Peziza hybrida*. The recent collections will undoubtedly enable us to prove or disprove this suspicion.

Probably the most important discovery in this region was the perfect stage of the sclerotial disease of the aspen trees. The imperfect stage, *Sclerotium bifrons*, has long been known and is so abundant in this section that it is a serious menace. Fully twenty-five per cent of the leaves of some of the trees were killed and it seemed to become worse as the season advanced. Dr. T. D. A. Cockerell suggested the name "ink-spot fungus" as an appropriate one for the sclerotial stage of the organism and it seems quite suitable since at this stage the fungus looks like a blot of ink.

While the sclerotial stage of the fungus has been known since 1890 and collected frequently in the Rocky Mountain states, so far as the writers were aware the perfect stage had never before been observed. Knowing that this should be a stipitate cup-fungus of the *Sclerotinia* type, special search was made for it. Persistence finally won and in a moist ravine near the University of Colorado Summer Camp the ascigerous stage was obtained in abundance. This occurred where the leaves had matted together and were kept constantly moist. Apparently the apothecia develop only after the leaves have lain for several seasons. Often the sclerotia fall out but more frequently they fall with the leaves and remain after the leaf has entirely decayed. Since this discovery was made, Professor H. H. Whetzel of Cornell University, who has made a careful study of this genus, reports having seen apothecia, of the fungus *Sclerotinia bifrons*, near Ithaca but, so far as can be discovered, these observations have not yet been published.

Each sclerotium produces from three to six or eight stipitate apothecia, the length of the stem varying with the conditions. The apothecia are about 1–1.5 mm. in diameter and the stem up to 4 mm. in length (PLATE 4). The whole ascophore is whitish or slightly yellowish. When mature, they puff profusely. Whether infection takes place directly from the germinating

ascospores has not been determined but since the sclerotia and apothecia occur at about the same time, it is likely that it does. A detailed study of the life-history of the fungus would furnish an excellent problem for some local research student.

Returning to Boulder on July 31, the party started the next morning for Laramie, Wyoming to attend the summer meeting of the western branch of the Botanical Society of America which was to be held at the University of Wyoming Summer Camp. Visiting botanists were lodged the first night in the luxurious quarters of the men's dormitory of the University. A banquet was held during the evening at which Dr. T. D. A. Cockerell was the chief speaker, and the next morning the entire delegation started for the summer camp, located at an elevation of 9,600 in the mountains of the Medicine Bow Range, and about forty miles distant from the University.

The mycological section was ably presided over by Professor Joseph C. Gilman of the State College at Ames, Iowa. The pathologists united with the mycologists since their interests were for the most part in common with them. The special interests of the collectors were diverse, the authors being concerned with the cup-fungi and the woody fungi while Professor A. O. Garret of Utah was rather expert in the collection of the rusts and smuts and quite familiar with the hosts in this region; the interests of other members of the section were more general. Consequently a large collection of rusts was made and will be eventually divided among the various collectors present. The work of the mycological section consisted largely of excursions into the field, supplemented by brief conferences in camp (PLATE 5) in which the collections of the day were reviewed.

The entire delegation of botanists joined in some of the expeditions under the guidance of Professor Aven Nelson. On one occasion cars were driven to Brooklyn Lake, a beautiful body of water located at an elevation of about 10,500 feet and constantly fed from the melting snow pocketed in the gulches on all sides. The party completely encircled the lake gathering many rare sub-alpine flowering plants while the mycologists collected a number of interesting fungi including parasites on these plants.

NEAR UNIVERSITY OF COLORADO SUMMER CAMP

During the last day of the meeting, a trip was made to the summit of Medicine Bow Range. Cars were driven as far as road conditions permitted and the latter part of the trip was made by some of the sturdy members of the party who had a likeness for climbing. Others botanized about the numerous mountain lakes of this region. One object of interest was the red snow caused by the alga, *Sphaerella nivalis*. The meeting closed on Sunday and the writers returned to their base at Boulder.

This first summer meeting of the Botanical Society was a decided success and the members present were unanimous in requesting that they be continued. Whether the meetings should be held in the same place each year or whether they should be rotated were matters which only the central organization can decide. The University of Wyoming Summer Camp is an ideal place for meetings such as this and the University extended a most cordial invitation to come again, if the society should see fit to do so.

After the return drive of 180 miles on August 4, the writers started early the next morning on a previously arranged excursion to the Black Hills of South Dakota at the invitation of Professor Junius Henderson who was especially interested in securing cycads from the Cycad National Monument. In spite of the fact that the drive was across the semi-arid plains of Colorado and Wyoming, a number of parasitic fungi were collected along the way. Camp was made the first night at Torrington, Wyoming and a few specimens collected at that place. The journey was resumed next morning as far as Edgemont, South Dakota where it was interrupted by a heavy rain said to be the first in this section for several months. The trip was continued the next morning as soon as the roads became passable and after a brief stop at Wind Cave tents were pitched at Legion Camp, elevation 6,500, amid a forest of mixed pines (PLATE 7).

Conditions in these pine forests were exceedingly favorable for collecting fungi especially because of the almost daily showers. The forest floor yielded an abundant crop of agarics and boletes. While stationed here, a drive was made through the Needles of South Dakota to Sylvan Lake and a number of fungi collected.

The work, however, was interrupted by rain. The following day, August 9, camp was broken and the return drive begun, stopping at intervals between Legion Camp and Wind Cave to collect. On one of these brief pauses, an unusually fine collection of *Hypomyces Lactifluorum* was secured. This fungus is a parasite on some species of *Lactaria* and so obliterates the gills that the specific identity of the host is determined with difficulty, if at all. The whole parasitized plant is unusually conspicuous since it assumes a brilliant orange red color which turns to purple as the host decays. After visiting the Cycad National Monument, camp was made for the night at Hot Springs, South Dakota. On August 10, the return was continued and after camping again at Torrington, the party arrived in Boulder on the afternoon of August 11.

After spending two days in caring for materials and exploring some of the nearby canons, an expedition was planned to Pikes Peak primarily in order to visit the Alpine Laboratory of the Carnegie Institution which is under the direction of Dr. F. E. Clements. Numerous stops were made and some very rich collecting grounds encountered between Sedalia and Colorado Springs by way of the foothills road which has been recently constructed. From Manitou the ascent was made, by means of the cog road, to Minnehaha, the site of the Alpine Laboratory, elevation 8,300 feet. Two very pleasant and profitable days were spent here and the writers were accompanied on one of these by Dr. and Mrs. Clements whose familiarity with the surroundings made it possible to obtain a large collection of cup-fungi and much data was acquired which will be of use in future work. The return to Boulder was made on August 16.

The senior writer after a few days in Denver returned to Boulder and spent an enjoyable evening with Dr. Elam Bartholomew so well known as distributor of Fungi Columbiani, as well as for other valuable mycological contributions. While Dr. Bartholomew was spending a few days in Boulder visiting his daughter, he and the junior author made a one day collecting trip to Tolland.

A short stay was next planned in Coal Creek Cañon at "El Joya del Monte," elevation 7,500 feet, the mountain home of

SCLEROTINIA BIFRONS

Mr. A. G. Seaver, brother of the senior writer. While here a drive was planned to the mouth of the Moffatt Tunnel, just above Tolland at an elevation of 9,000 feet, and a number of collections made.

Returning to Boulder on August 27, the last long expedition of the summer was arranged to Grand Lake. This is the largest lake in Colorado and one of the few lakes of its size to be found at this high altitude, 9,000 feet. The writers made the drive from Boulder to Grand Lake between 8:00 P.M. and midnight by way of Fall River Pass which ascends to an elevation of about 12,000 feet and well above timber line. The two days spent at the lake yielded good results.

After spending a few days in Boulder packing and shipping materials, the senior writer returned to Denver and started on September 7, for the East, while the junior author, accompanied by Mr. M. O. Jung, proceeded to the western slope and collected in the Ohio Creek region, near Gunnison; thence to Grand Mesa where collections were made over a period of eight days. Nearly six hundred collections of fungi were taken including many valuable cup-fungi, one of the chief objects of quest, and which will be of vast importance in working up a second volume of the North American Cup-fungi which is now in course of preparation. Sufficient material was obtained so that many duplicates can be made for exchange, especially with foreign countries, and thus add still more to our already large collection of this group of fungi and, at the same time, help to correlate European and American forms.

In general the summer of 1929 was a poor one for collecting fungi. The early summer was cold and extremely dry which no doubt exercised some influence on the fungi in general. After the first week in July, daily summer showers occurred. The junior author records the summer's collections of polypores as the poorest in the past five years. The Grand Mesa region was, however, better watered than the mountainous region farther east. Good collections of polypores and other fungi were made here.

The writers wish to express their hearty thanks to Professors Ramaley, Junius Henderson, and T. D. A. Cockerell of the

University of Colorado; also Dr. W. E. McCourt and members of the Staff of the University of Colorado Summer Camp; to President A. G. Crane, Professor Aven Nelson and members of the staff of the University of Wyoming; and to Dr. and Mrs. F. E. Clements of the Alpine Laboratory for their hospitality and cooperation in the prosecution of this work.

THE NEW YORK BOTANICAL GARDEN
UNIVERSITY OF COLORADO

EXPLANATION OF PLATES

All photographs accompanying this article were made by the junior author.

PLATE 1. Camp site in Middle Boulder Canon, elevation about 10,000 feet. The cabin in the lower left-hand corner was occupied by the authors of this article July 23–26, 1929.

PLATE 2. Middle Boulder Canon, showing Arapahoe Peak (upper figure) and Diamond Lake (lower figure). The Lake is located about 1,000 feet above the camp site shown in PLATE 1.

PLATE 3. Aspen grove and mycological base near University of Colorado Summer Camp, where the perfect stage of the ink-spot fungus was collected.

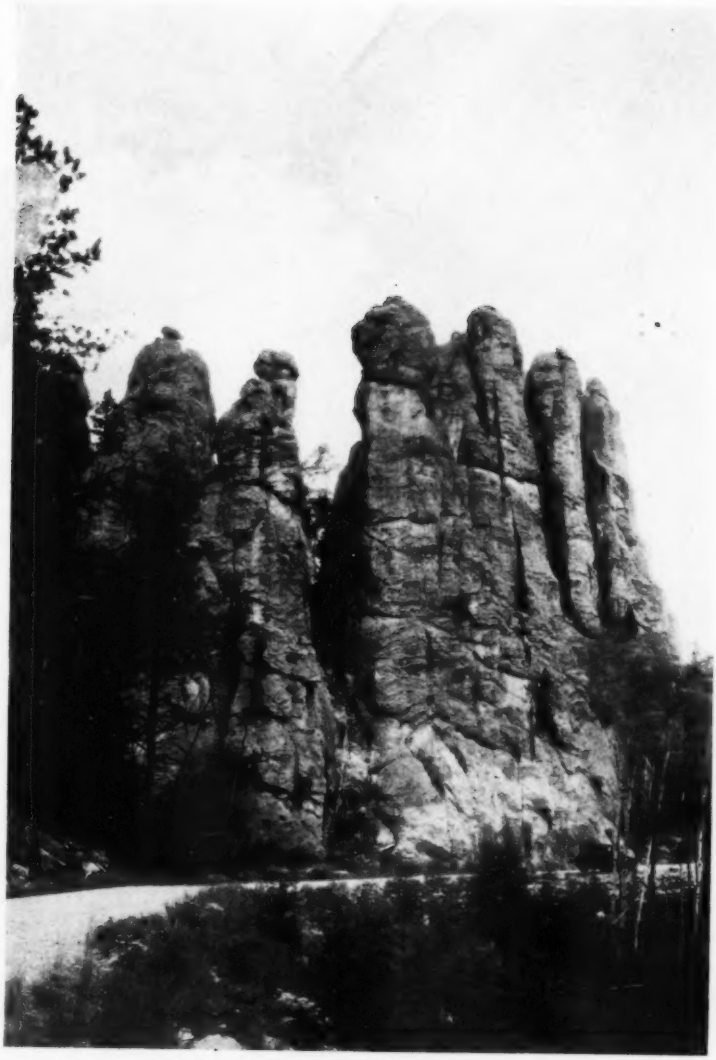
PLATE 4. *Sclerotinia bifrons*. Near the center a branch of an aspen, slightly reduced, showing healthy leaves and those infested with the ink-spot fungus (*Sclerotium bifrons*). Note the zonate markings of the infected leaves. At the right three clusters of apothecia growing from the old sclerotia and enlarged about five times. At the left drawing of an ascus and the spores, much enlarged.

PLATE 5. University of Wyoming Summer Camp in the Medicine Bow Mountains, elevation 9,600 feet, showing the newly erected assembly room in which the meetings of the Botanical Society of America were held the summer of 1929.

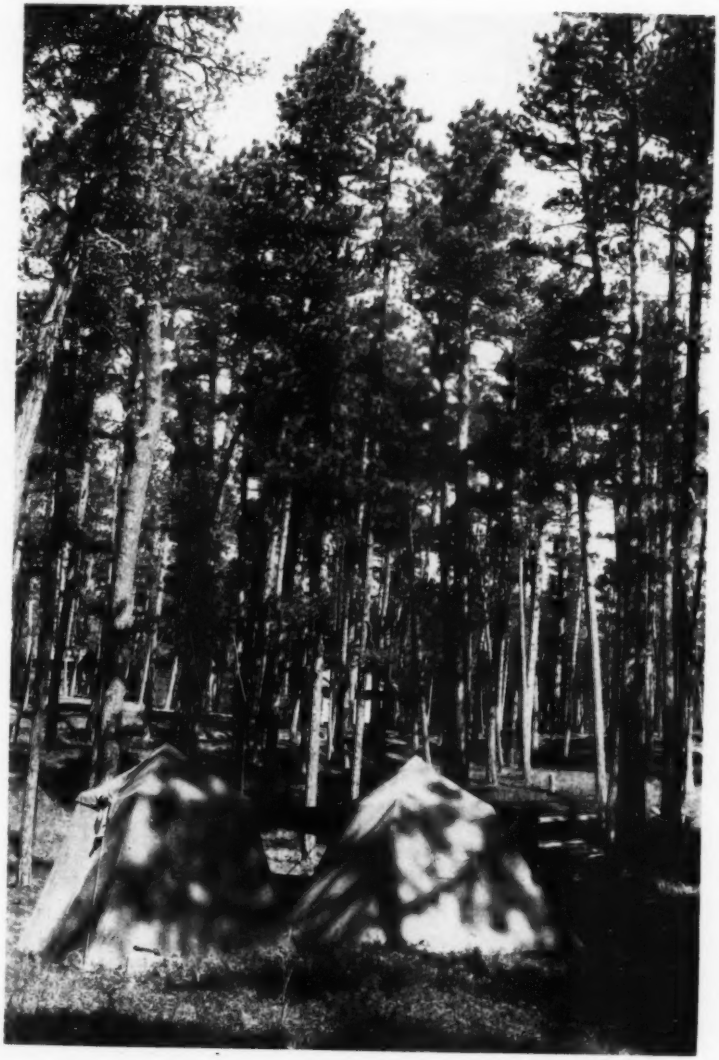
PLATE 6. Drive through the Needles of South Dakota showing the spire-like formation of the rocks.

PLATE 7. Mycological base in the Black Hills of South Dakota located in Legion Camp, elevation 6,500 feet, and forests of mixed pines.

UNIVERSITY OF WYOMING SUMMER CAMP



NEEDLES OF SOUTH DAKOTA



BLACK HILLS OF SOUTH DAKOTA

BREEDING ALBINISTIC STRAINS OF THE MONILIA BREAD MOLD

B. O. Dodge

(With Plates 8 and 9 and 1 Text Figure)

Introduction

Mutations are frequently reported as appearing in laboratory cultures of *Aspergillus*, *Alternaria* and other species of the imperfect fungi. In some instances certain stimuli or environmental conditions are conducive to variation, yet no one has explained why these particular fungi are so prone to mutate. Clearly if the cause of the sudden alteration in growth forms is some change in the chromosome complex, it must have been effected in nuclei long since haploid. The custom of propagating the mutants asexually does not serve to tell us very definitely whether it is the cytoplasm, the nuclei as a whole, or the individual chromosomes which are the units involved. Harder (**9**) has experimented with mycelia of certain Hymenomycetes and proved that in some instances the cytoplasm itself is effective in determining the type of mycelial growth. The situation is one which serves to emphasize the need of knowing the full life history of the mutating fungus so that it can be cultured and reproduced sexually. Nuclear fusions and reduction divisions provide the opportunity for bringing together and redistributing factors of inheritance. If any such elements are involved in the origin of an *Aspergillus* mutant, for example, a series of cultures from single ascospores obtained by propagating the mutant sexually ought to reveal the fact.

A bisexual mycelium of *Neurospora tetrasperma* frequently forms a unisexual conidium (**5**). The mycelium developed from this conidium will never produce ascocarps. The mycelia from normal conidia of the parent mycelium do so readily. The change is simply due to the unequal distribution of nuclei in the hyphal branches forming the conidia. Normal conidia receive two kinds of nuclei, the abnormal only one kind, as

regards their sex. The sex of each nucleus is determined in the reduction divisions in the ascus. No change of sex occurs in somatic divisions. Suppose the unequal distribution of nuclei had taken place soon after the bisexual germinating ascospore had been planted at the center of a plate culture; a whole sector of the mycelium as it grew out would have been unisexual. Transfers from this sector would be quite unlike the parent form culturally and sexually. This is a sort of mutation easily explained only because something is known of nuclear behavior (**3**).

The albinistic strains of *Monilia sitophila* reported recently (**6, 7**) could perhaps be regarded as true mutations. Their chief interest, however, lies in the fact that their failure to produce conidia in the fashion characteristic of the species seems to be due to certain inherited factors which regularly segregate in the reduction divisions. An analysis of the mycelia from which the sterile albinistic strains were developed by sexual reproduction suggests that somatic changes may have been instrumental originally in bringing about conditions which were first made manifest when ascospores from the mating were germinated.

Formerly in breeding work involving animals and higher plants one was not much concerned as to just where in the divisions of the mother-cell segregations of the factors of inheritance occurred. Dealing with the progeny of large numbers of mother-cells in mass or as populations it really made little difference. The fungi, particularly the heterothallic ascomycetes and basidiomycetes are now being studied more and more from the standpoint of genetics. The peculiarities of the ascus, the promycelium, and the basidium are such that the products of the reduction divisions of individual mother-cells can be singled out and mated together or inbred in a way absolutely precluded in a case of higher plants and animals. This gives to the question of certain details of segregation an added interest.

In the course of a study on giant ascospores (**6**) it was found that in a normal ascus of *Neurospora crassa* the eight spores alternate four and four as to their sex, instead of two and two as had been previously reported (**14**) for *N. sitophila*. It was pointed out (**7**), however, that in certain strains of the latter

species also the sex factors are frequently segregated in the first division so that the spores alternate four and four in the ascus as to their sex. The culture experiments which furnished the evidence for these conclusions, and the methods by which albinistic strains were obtained will now be described in more detail.

ARLINGTON STRAINS OF NEUROSPORA SITOPHILA

Among the "Arlington" strains of *Neurospora sitophila* cultured by Shear and Dodge (**13**) were two strains which were kept as stock cultures by the writer because they always produce perithecia abundantly when grown together. They are readily distinguished from each other by the color of their conidial masses. "Arl.6," which is a haplont A, produces the salmon pink or light orange colored conidia. "Arl.10" is a haplont B. It is somewhat lighter in color perhaps because it usually develops fewer conidia. It produces more of the dark colored bodies which have been referred to as sclerotia or bulbils. Both strains are presumed to have been derived from single ascospores originally; each has since been transferred many times. The two Arlington strains or clons are the ones cultured to obtain the results reported. Their peculiarities, particularly those of Arl.10, will be described more fully later in this paper.

METHODS OF CULTURE

The culture methods employed have been fully described (**4, 13**). Heretofore it had been observed that ascospores of this species rarely germinate at room temperatures. The discovery that several spores which had been isolated from one ascus had germinated in the interval during which they had been set aside to allow conidia to germinate before the ascospores were heated, suggested a change in the procedure. Taking greater precautions to exclude conidia, the ascospores that germinate without heating are first transferred, and then the remainder are stimulated to grow by heating them. It is only by chance even then that one gets all eight spores from an ascus to grow.

ALBINISTIC RACES OF NEUROSPORA SITOPHILA

Ascus no. 56 was the first one from which cultures were obtained from all of its spores. This set of eight cultures has been referred to briefly in another connection (**6**, p. 230). The spores had been isolated one by one so that the position which each had occupied in the ascus was known with certainty. The germinating spores were first transferred to agar in petri dishes. Spores nos. 1, 2, 3 and 4 were put in one plate and nos. 5, 6, 7 and 8 in another. The germ tubes were allowed to grow and branch until it was possible to obtain two sets of tube cultures by transferring tip ends of young hyphae. The ascospores themselves were not transferred. Each plate culture therefore still contained four young haplonts derived from four ascospores from the one or the other end of the ascus. If no perithecia developed in either culture it would be positive proof that all four spores in one end of the ascus were alike as to sex. If perithecia did finally develop, nothing would be proved except that in some way two sexually different spores had been introduced into the same culture. The tube cultures were numbered consecutively 1 to 8 to indicate the original positions of the spores in the ascus. They were then placed in a tube rack in the same order. This little detail was probably the one thing that brought out the very striking differences which the cultures showed after they had been incubated at 27° C. for two days. Cultures nos. 1, 2 and 5, 6 had already developed an excessive growth of whitish aerial hyphae, but no conidia, while cultures nos. 3, 4 and 7, 8 showed that many salmon-pink conidia were being formed. This was interpreted to indicate an alternation in pairs as to sex as described by Wilcox (**14**). The parent strains Arl.6 and Arl.10, it will be remembered, are readily distinguished from each other by the color and extent of their conidial masses.

Assuming that haplonts nos. 1, 2 and 5, 6 would eventually develop conidia, the cultures were again incubated for two more days. At the end of this time the differences previously noted appeared to be even more marked. Four of the cultures still showed no conidia. The illustration, PLATE 8, was reproduced from a hand colored photograph of this set made when the

cultures were four days old. A third set of 8 cultures was made a few days later. After many months the three sets of cultures still present a very striking picture of an alternation in pairs as regards production of orange-colored conidia. The aerial hyphae of mycelia nos. 1, 2 and 5, 6 produce few if any conidia. Without knowing their history it is a question whether one would recognize these mycelia as belonging to a species of *Monilia*, much less to *Monilia sitophila*. There is sometimes a tendency to form constrictions along the tip ends of the aerial hyphae, but, if any, there are certainly very few real conidia dislodged. The method of branching as the hyphae grow against the side of the test tube is suggestive, perhaps, of a *Monilia*. Under certain conditions the tufts or mats of aerial hyphae formed at the top of the agar slant have a pale yellowish color, but, in general, they are only faintly tinted. These strains will be referred to as albinistic for convenience, although, as noted previously, their real interest lies more in the fact that they are practically sterile as regards production of conidia.[1] On the other hand they have provided in their sclerotia or bulbils, a very efficient means of asexual reproduction. The great quantities of the dark colored bodies is one characteristic of the strains. This would enable one familiar with this peculiarity of species of *Neurospora* to recognize the mycelia as belonging to that genus.

The element of pink which appears even in old cultures of the parent strains Arl.6 and Arl.10 is not evident in old cultures of haplonts nos. 3, 4 and 7, 8 from ascus no. 56. Their masses of conidia are golden in color. Such strains of the fungus may well have served Léveillé as a type for his *Oidium aurantiacum* or Kitasima for his *Monilia aurea* (**13**). These conidial strains or clons also produce many little sclerotia, but they are not dark colored at first. Later on as the culture begins to dry down the sclerotia darken and they then look much like the bodies produced in cultures of the albinistic clons. The first necessity of the species is the production of conidia to spread the fungus far and wide. When an abundance of these reproductive structures

[1] To avoid digression, and confusion of the main issue at this point, a discussion of the differences between monilioid conidia, *Oidium*-like fragments of hyphae and true microconidia will be taken up in a later paragraph on microconidia.

has been provided then sclerotia are formed to maintain it over unfavorable periods. The albinistic strains develop sclerotia at once.

It has been shown (**14**) that the spindles of the first and second nuclear divisions in the ascus of *Neurospora sitophila* are placed longitudinally. No evidence has been found to suggest that there is any material change in the location of the four nuclei resulting from the second division. They lie far apart in a row on the long axis of the ascus, and separated from each other by a vacuolate cytoplasm. Since the spindles of the third division are transverse there is necessarily a slight readjustment of the spores after they are cut out to bring them into line. Spores nos. 1 and 2 of ascus no. 56 must have contained sister nuclei resulting from the third division. They would possess the same sets of factors of inheritance whether segregation occurs in the first or in the second nuclear division in the ascus. It is the same with the spores of the pairs 3 and 4, 5 and 6, 7 and 8. If the visible differences exhibited by the alternating pairs of haplonts were due to factors of inheritance which are regularly segregated, and not to some fortuitous set of environmental conditions prevailing at the time, segregation of these factors must have occurred in the second division in the ascus and not in the first, otherwise the ascospores would have alternated four and four as regards the production of conidia.

Neurospora sitophila has been proved to be heterothallic. There are only two sexes, and a particular mycelium will be of one or the other of these two sexes. This is not saying that certain pairs of strains are not more fertile or compatible than other pairs when grown together. In fact this is one of the notable things observed in culturing the species as will be pointed out later. Occasionally a single ascospore culture will develop a few large bodies resembling perithecia (**13**). Many of these structures in old cultures have been examined but as yet no ascospores have been found in them. It is evident that a haploid mycelium can occasionally develop the framework of a perithecium without being paired with a mycelium of the opposite sex. It is possible that by special treatment these bodies could be induced to develop further and even produce spores. From

a genetic standpoint this would be of the greatest interest. Obviously any abnormal ascospore which acquired by accident two nuclei of opposite sex at its delimitation would give rise to a homothallic mycelium which would of itself be capable of producing perithecia. No doubt further study of giant ascospores will result in the discovery of just such strains. To determine the sex of 8 haplonts derived from a particular ascus it is only necessary to grow each one in a culture with the same "tester" strain. In order that there could be no question on this point the custom of growing the haplonts in pairs in all possible combinations was adhered to. Duplicates were not always made however.

The 8 haplonts derived from ascus no. 56 were grown in pairs in all possible combinations. It may be well to consider in advance what this would involve. First, there would be six combination cultures in which two albinistic strains would be grown together. Second, there would be sixteen cultures in which one albinistic strain would be grown with a strain which produces typical orange-colored conidia. Third, there would be six other combination cultures, both strains of which produce conidia. Furthermore if the factors for sex and the factors for conidia, were segregated at the same time, namely in the second division, then the only cultures which would develop perithecia would be the sixteen containing one albinistic and one conidial strain.

Should, however, the factors for sex be segregated in the first division so that the spores alternate four and four in the ascus as regards their sex, it is clear that one pair of albinistic haplonts, nos. 1 and 2 would be of one sex and the other pair, nos. 5 and 6, would be of the opposite sex; conidial strains nos. 3 and 4 would be of opposite sex to that of conidial strains nos. 7 and 8. Perithecia could be expected in sixteen cultures. Four of these cultures would each contain a pair of albinistic strains of opposite sex; four cultures would each contain a pair of conidial strains of opposite sex; eight cultures would contain one albinistic and one conidial strain of opposite sex. The possibility of developing new races which could be propagated indefinitely sexually depended on where the sex factors separated.

The results of the matings are shown in TABLE I and prove conclusively that the two sets of factors had segregated independently.

TABLE I

RESULTS OBTAINED BY GROWING IN ALL POSSIBLE COMBINATIONS THE EIGHT HAPLONTS DERIVED FROM ASCUS NO. 56 OF *Neurospora sitophila*

Haplonts are numbered in the order in which the ascospores were disposed in the ascus. The sign + indicates that perithecia were formed. The sign − indicates that no perithecia were produced.

	1	2	3	4	5	6	7	8
1	−	−	−	−	+	+	+	+
2	−	−	−	−	+	+	+	+
3	−	−	−	−	+	+	+	+
4	−	−	−	−	+	+	+	+
5	+	+	+	+	−	−	−	−
6	+	+	+	+	−	−	−	−
7	+	+	+	+	−	−	−	−
8	+	+	+	+	−	−	−	−

The table shows that haplonts nos. 1, 2, 3 and 4 were all of one sex, and haplonts nos. 5, 6, 7 and 8 were of the opposite sex. This means that the sex factors had separated in the first division. Four cultures, 56(1 + 5), 56(1 + 6), 56(2 + 5) and 56(2 + 6), each containing two albinistic mycelia, produced perithecia very quickly and abundantly. The eight cultures containing one albinistic and one conidial strain each, and which produced perithecia, were the following: 56(1 + 7), 56(1 + 8), 56(2 + 7), 56(2 + 8), 56(3 + 5), 56(3 + 6), 56(4 + 5), and 56(4 + 6). Four cultures, 56(3 + 7), 56(3 + 8), 56(4 + 7) and 56(4 + 8), containing two conidial strains, also produced perithecia.

Since it is not known whether the two sexes referred to are differentiated as male and female, we shall for convenience continue to call one sex A and the other sex B (**13**). All unisexual mycelia of one species which have the sex A are haplonts A. Mycelia having the opposite sex are haplonts B. In the diagrams

(FIG. 1) ascospores which have the sex A are uncolored, and those having sex B are colored black. A spore which carries the factors for conidia is dotted either black or white depending on the color of the background. The four kinds of spores contained in an ascus of the general type of ascus no. 56 are represented diagrammatically in FIG. 1, *C*.

ALBINISTIC RACES MATED

Whether or not the mycelia would breed true to type could only be told by growing ascospores produced in perithecia from the various matings. It was evident that cultures containing certain pairs of albinistic mycelia were developing perithecia. Would the ascospores of the next generation produce albinistic strains in turn? Due to the fact that the cultures were being incubated at 27°, the mycelia grew rapidly. Perithecia began to appear first in culture 56(2 + 6) on the third day. At the end of 11 days mature ascospores were being discharged. One ascus designated as 56(2 + 6)a to indicate its parentage, was selected. The 8 spores were isolated one by one in order and germinated. The mycelia thus obtained were numbered consecutively 1 to 8 to correspond to the order in which the spores had been disposed in the ascus. The haplonts were then grown together in pairs in all possible combinations and in duplicate. The results of the matings are shown in TABLE II.

Haplonts nos. 1, 2 and 5, 6 were found, as indicated in the table, to be of the same sex, and haplonts nos. 3, 4 and 7, 8 were also alike sexually but of the opposite sex. The spores thus alternate in pairs, so that segregation of the sex factors in this case must have occurred in the second nuclear division. Such an ascus is diagramed in FIG. 1, *D*.

These cultures show that the albinistic character possessed by the parent strains nos. 2 and 6 from ascus no. 56 has been retained in the offspring. That this is a rather fixed character has been further demonstrated by germinating many ascospores of the new generation. Spores from culture 56(2 + 6) were sowed on the surface of agar in petri dishes. Fifteen different mycelia were obtained from single ascospores which happened to germinate without heating. These haplonts were all albinistic. The

spores of the original sowing which did not germinate were then heated moderately, and many more were thus stimulated to grow. Ten germinating spores were selected at random. Their

TABLE II

RESULTS OBTAINED BY GROWING IN ALL POSSIBLE COMBINATIONS THE EIGHT ALBINISTIC HAPLONTS DERIVED FROM ASCUS No. 56(2 + 6)a

The haplonts are numbered in the order in which the ascospores were disposed in the ascus.

	1	2	3	4	5	6	7	8
1	−	−	+	+	−	−	+	+
2	−	−	+	+	−	−	+	+
3	+	+	−	−	+	+	−	−
4	+	+	−	−	+	+	−	−
5	−	−	+	+	−	−	+	+
6	−	−	+	+	−	−	+	+
7	+	+	−	−	+	+	−	−
8	+	+	−	−	+	+	−	−

mycelia were also all albinistic. Other ascospores were subjected to a very much higher temperature, well toward the maximum, evidently, for only a few spores germinated as a result. Thirteen haplonts were selected. Again all proved to be albinistic. Tufts of their mycelia were shaken in sterile water and plates were poured in the ordinary way. When three loops of the water were included only one colony was developed. This had originated from a fragment of mycelium and not from a conidium. Another plate which contained three loops of a suspension from the conidial strains 56.8 developed a great many colonies. Albinistic mycelia have been grown on hard corn meal agar, dextrose agar, potato dextrose agar, sterilized bread in flasks, on loaves of bread under bell jars and on potato plugs. In some cases the mycelium possesses more of the pink coloration than it shows on corn meal agar. If conidia were produced they were not noticeable. No evidence has been obtained to show that the kind of medium makes any material difference.

It is scarcely to be expected that the albinistic strains are absolutely sterile. Conditions may be found under which some monilioid conidia will be formed normally.

MATING TWO CONIDIAL STRAINS FROM ASCUS NO. 56

When, for example, strain 56.4 is paired in culture with strain 56.8, there will be two conidial strains of opposite sex growing together. Each strain produces its own conidia and naturally such a culture will show an abundance of conidia. In order to learn whether ascospores from this kind of culture would be arranged in the ascus, two and two or four and four as to their sex, spores from one ascus designated 56(4 + 8)a, to indicate its origin, were isolated. Only five spores germinated. These were nos. 1, 3 and 4 from one end of the ascus, and nos. 5 and 7 from the other end. Tube cultures were obtained in the usual way, after which the haplonts were grown together in pairs in all possible combinations. The results were conclusive in spite of the fact that three of the eight haplonts were missing. All of the mycelia produced conidia. Haplont 1 was proved to be of opposite sex to that of haplonts 3 and 4. Haplonts 5 and 7 when grown together developed perithecia, showing that they were of opposite sex. Again segregation of the sex factors had occurred in the second division, for the spores alternated in pairs as to their sex (FIG. 1, *E*). A checkerboard diagram to show the results of these matings would be of the same type as that shown in TABLE II.

After culture 56(4 + 8) had matured sufficiently so that a spore print had been formed on the side of the tube, sowings of ascospores were made in the manner described in connection with culture 56(2 + 6). Thirty single ascospore mycelia were obtained. All of the cultures showed the same great abundance of golden colored conidia. Certainly the segregations that had occurred in the two generations had in some way served to purify these conidial strains. Their conidia show the pink coloration only in young cultures. When strain no. 7, for example, is grown on a loaf of bread under a bell jar and strain no. 2 from ascus no. 56 is grown under similar conditions, the pictures presented suggest two entirely different species of mold.

The results of the experiments which have been described indicate that ascospores obtained by mating two albinistic races in turn develop albinistic mycelia. When two conidial strains are mated the mycelia from their offspring ascospores will produce conidia prolifically. In other words, these strains breed true for conidia as far as they have been tested out.

MATING AN ALBINISTIC RACE WITH A CONIDIAL RACE

It remains to learn what will be the results when an albinistic strain is crossed with a strain which bears conidia normally. Culture 56(2 + 7) was selected for this experiment. Haplont no. 2 from ascus no. 56 is albinistic; no. 7 from the same ascus bears conidia. The ascus designated as 56(2 + 7)a was isolated from a perithecium of the culture. The 8 spores were carefully separated and germinated. The tube cultures obtained were numbered consecutively 1 to 8 to show the order of the spores in the ascus. After having been incubated for two days it was evident that segregation of the factors for conidia had again occurred in the second nuclear division in the ascus. Four of the spores, nos. 3, 4 and 7, 8 had developed albinistic mycelia, and four spores, nos. 1, 2 and 5, 6 had developed mycelia which were already cutting off conidia. The eight mycelia were grown together in pairs in all possible combinations. The results were again conclusive. Four genotypically different kinds of spores had been obtained from a single mother-cell. Spores nos. 1, 2, 3 and 4 proved to be all alike sexually and were of sex B. Spores nos. 5, 6, 7 and 8 were of sex A. Sex factors had been segregated in the first nuclear division in the ascus and the factors for conidia had separated in the second division as was the case with ascus no. 56 (FIG. 1, *C*).

If segregations are always regular, as shown in ascus 56(2 + 7)a when albinistic and conidial strains are mated, every ascus should produce four albinistic and four conidial ascospores, and random selections of ascospores from a spore print should give about equal numbers of the two kinds. Of 77 haplonts obtained from single spores, 49 developed conidia and only 28 were albinistic. In the four different sets of cultures made at various times the results were: 11 conidial, 22 albinistic; 16 conidial, 1 albinistic;

12 conidial, 1 albinistic; 10 conidial, 4 albinistic. Experience has shown that if one selects only the germinating spores at random the results are apt to vary widely from the expectancy. In case of one back-cross mating (**4**, p. 10) 11 small germinating ascospores were transferred. The haplonts thus obtained were all of sex A. The same experiment was repeated and of the 28 mycelia isolated 20 were of sex A and 8 were of sex B. Further work should be done before reaching the conclusion that the mating of albinistic and conidial strains always results in segregations of the type shown by asci nos. 56 and 56(2 + 7)a.

OTHER ASCI FROM CULTURE ARL.6 + ARL.10

The deduction naturally to be made on the basis of the results of the four experiments with individual asci would be that when "pure line" strains of opposite sex are mated, there being no segregation of factors for conidia, the sex factors are regularly segregated in the second division in the ascus. Further, when albinistic strains are crossed with conidial strains the sex factors are segregated in the first division and the conidial factors in the second. This does not explain how it happened that in mating strains Arl.6 and Arl.10 there should have occurred the very definite segregation of conidial factors as shown by ascus no. 56, because both parent mycelia produce conidia. Although no. 56 was the first ascus from which all eight spores had been isolated in order and germinated, a sufficient number of spores from five asci, nos. 44, 45, 46, 48, and 50 had been germinated to show, when examined later, that segregation of conidial factors had occurred. From one to three of the haplonts obtained from each ascus were albinistic. The fact that these haplonts were not producing conidia was not observed at the time the cultures were first obtained. The spore numbers were too small to be of value to determine how the two sets of factors had been segregated except in case of ascus 46. In this ascus the sex factors had clearly been separated in the first division and the conidial factors in the second division. In order to obtain further evidence regarding the nature of asci produced in cultures of Arl.6 + Arl.10 several asci were isolated from perithecia selected from a new culture. At least six spores from

each of eight asci germinated. The characteristics of the haplonts from each ascus and the type of segregation will now be described briefly.

All eight spores from ascus no. 57 germinated but none of their mycelia, however, proved to be albinistic. They were all forming conidia very rapidly by the third day. The eight haplonts were grown together in pairs in all possible combinations to determine the sex of each one with certainty. The results showed that, as was the case with ascus no. 56(4 + 8)a where all of the haplonts produced conidia, segregation of the sex factors had occurred in the second division, so that the spores had alternated two and two as to sex (FIG. 1, *E*). Ascus no. 62 proved to be of the same type as ascus no. 57. The eight mycelia derived from ascus no. 58 also produced conidia. In this case it was proved that the sex factors had separated in the first division. The four spores from each end of the ascus were all alike as to sex. Ascus no. 60 was of the same type as no. 58 (FIG. 1, *B*).

Ascus no. 61 showed an entirely new type. The 8 spores had been carefully isolated and numbered consecutively 1 to 8. Mycelia nos. 1, 2, 3 and 4 were albinistic and mycelia nos. 5, 6, 7 and 8 produced the golden colored masses of conidia. By growing the haplonts in pairs in all combinations it was found that nos. 1, 2 and 5, 6 were of sex A and nos. 3, 4 and 7, 8 were of sex B. The spores had alternated in the ascus four and four for conidial factors and two and two as to sex (FIG. 1, *A*). Conidial factors had separated in the first division and sex factors in the second. Asci nos. 63 and 65 were like ascus no. 61 in this respect, but ascus no. 64 was like ascus no. 56 where the spores had alternated two albinistic and two conidial, and four of one sex and four of the opposite sex. A summary of the results of all the experiments where five or more haplonts were obtained from an ascus is given in TABLE III. The sex of each haplont, determined by mating it against a tester strain, is designated as A, or B. If a haplont produces conidia this fact is denoted by the sign +. The sign − signifies that the haplont is albinistic.

Six of the 14 asci reported on in detail in the table show segregation of the sex factors in the first nuclear division, while

TABLE III

SEGREGATION OF FACTORS FOR SEX AND FOR CONIDIA IN FOURTEEN ASCI OF *Neurospora sitophila*. PERITHECIA FROM CULTURES OF THE MATING ARL.6 + ARL.10

First half (S) of each double column shows the sex, A or B, of the particular haplonts which were obtained by germinating spores from that ascus. The second half (C) of each double column shows the production (+) or failure (−) of production of conidia by individual haplonts.

Ascus No.	46		56		56 (2+6) a		56 (2+6) b		56 (2+7) a		56 (4+8) a		57 [2]		58		60 [2]		61		62 [2]		63		64		65	
Spore No.	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
1	A	+	B	−	B	−			B	+	A	+	A	+	B	+	A	+	A	−	B	+	A	−			B	−
2			B	−	B	−	B	−	B	+			A	+	B	+	A	+	A	−	B	+	A	−			B	−
3	A	−	B	+	A	−	A	−	B	−	B	+	B	+	B	+	A	+	B	−	A	+	B	−	A	−	A	−
4	A	−	B	+	A	−	A	−	B	−	B	+	B	+	B	+	A	+	B	−	A	+	B	−	A	−	A	−
5	B	+	A	−	B	−	B	−	A	+	A	+	A	+	A	+	B	+	A	+	B	+			B	−	B	+
6			A	−	B	−			A	+			A	+	A	+	B	+	A	+	B	+			B	−	B	+
7	B	−	A	+	A	−	A	−	A	−			B	+	A	+			B	+	A	+	B	+	B	+		
8	B	−	A	+	A	−	A	−	A	−	B	+	B	+	A	+			B	+	A	+	B	+	B	+	A	+

[2] The exact order was not determined except that it was known from which half of the ascus each spore was taken.

these factors had separated in the second division in 8 asci. Segregation of conidial factors had occurred in the first nuclear division in three asci, and in the second division in four asci.

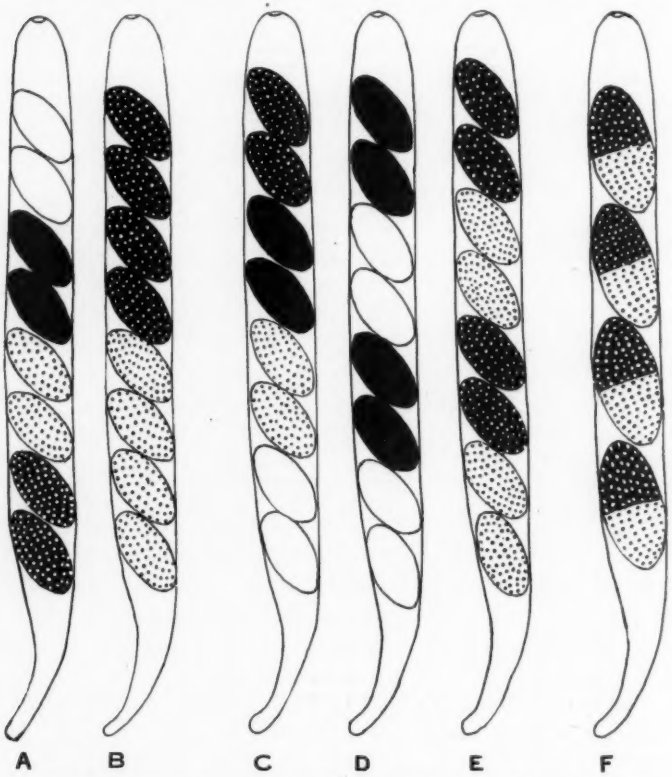


FIG. 1. Diagrams of asci showing that factors for sex and for conidia segregate independently in either the first or the second nuclear division. A–E. *Neurospora sitophila*. A. Ascus no. 61; B. Ascus no. 58; C. Ascus no. 56(2 + 7) a, same general type as no. 56; D. Ascus no. 56(2 + 6)a; E. Ascus no. 62; F. Normal ascus of *Nenrospora tetrasperma*. (See text for further explanation.)

Albinistic strains were obtained from other asci from which fewer than five spores germinated. These results are not included in the table. For example two spores from each end of ascus no. 45 mentioned previously were the only ones which

germinated. The two from one end produced albinistic mycelia while the two from the other end gave conidial mycelia. It happened that the conidial haplonts were alike as to sex and opposite to that of the two albinistic haplonts. It would be impossible to say without a fifth spore where either set of factors was segregated. Only spores nos. 1, 2, 3 and 4 were isolated from ascus no. 59 and germinated. Nos. 1 and 2 were found to be of sex A and nos. 3 and 4 were of sex B. The four haplonts all produced conidia. This ascus may have been either like ascus no. 61, in which case the four missing haplonts would have been albinistic; or no. 59 may have been like ascus 56(4 + 8)a where all of the haplonts produced conidia. A fifth spore would have determined the type. The results shown in the table indicate that the factors for sex and the factors for conidia segregate independently. No evidence has been found as yet to prove that both sets of factors can segregate in the same nuclear division. It would be strange, however, if such a type of segregation could not be found in view of the great variation in this regard already discovered.

Wilcox (**14**, FIG. 2) found four different arrangements of the spores in the asci she studied, but in each case each end of the ascus contained first a pair of spores of one sex, then a pair of the opposite sex. The writer has not happened to find an ascus in which the four spores at the middle, *i.e.*, spores nos. 3, 4, 5 and 6 were all of the same sex. No doubt such arrangements do occur frequently. The same would be true in case of a "hybrid" ascus like no. 56 where factors for conidia are segregated in the second division.

The two types of segregations illustrated in connection with asci nos. 56 and 61 appear to bring about exactly the same result, namely, the production of four different kinds of spores, two of each kind in each ascus. It should be noted however that the relationship of the haplonts that can be mated to produce perithecia is not always the same in both cases. Haplonts of opposite sex from ascus no. 56 are always second cousins, basing the relationship on the number of divisions subsequent either to nuclear fusion or to segregation. There will be 16 possible matings between second cousin individuals which will give

positive results. In case of haplonts from ascus no. 61 there will be eight possible matings between first cousin haplonts which will result in the production of perithecia. The other 8 productive matings will be between second cousin haplonts. "Pure line" albinistic as well as conidial strains can be obtained by inbreeding haplonts from ascus no. 61 only by mating first cousin haplonts. The fusion nucleus in an ascus from a second cousin mating of haplonts from ascus no. 61 will always be heterozygous for conidia.

ALBINISTIC RACE BY SALTATION

Edgerton (**8**) was among the first to note the effect of the environment on variations in the cultural characters of fungi. Some of the changes described were recognized for what are now regularly called mutations or saltations, as distinct from changes merely transitory, holding only so long as the new environment is maintained. Brown (**4**), although he erroneously attributed the coining of the word saltation to Stevens, has discussed briefly the use of the terms saltation and mutation to denote the sudden permanent changes in the morphological and physiological characters of fungi which occur in cultures. The art of growing fungi from single spores is so commonly practiced that one is better able than formerly to evaluate results reported by students of fungous variants. This subject will be covered in another paper where the behavior of strain Arl.10 described below will be compared with mutations or saltations previously reported by others.

The four conidial haplonts obtained from ascus no. 56 are typical for the species *Monilia sitophila*, and they resemble more the Columbia University and Cooley strains (**13**). In analyzing the parent strains to find out if possible, why some of the ascospores from the combination Arl.6 + Arl.10 produce albinistic mycelia, the purity of the stock cultures must first be established. One or the other might contain two mycelia, one of which is albinistic. Both being of the same sex, the mixture would not be manifest under the conditions of ordinary culture work. Again, one of the parent mycelia may have mutated previously and given rise to an albinistic hyphal branch which did not

produce conidia. In making new cultures by transferring conidia entangled in bits of mycelium the mixture would be continued from culture to culture. In either case there would be a chance when the two parent strains were grown together that the two nuclei which come to fuse in the young ascus would be unlike as to the factors for conidia which they bore. Such an ascus would produce four albinistic and four conidial ascospores. If, however, the perithecium arose from mycelial branches both of which produced conidia, no albinistic ascospores would be formed. The cells of the mycelium, ascogonium and, no doubt, those of the antheridium have more than one nucleus. This makes the situation more complicated than would be the case were each cell uninucleate. As it stands there is no guarantee that all of the asci in a perithecium have the same inheritance. They might be unlike with respect to their factors for conidia alone. All of the ascospores in five of the asci included in TABLE III produced conidial mycelia, but no record was kept as to whether the asci in any single perithecium were all alike in this respect. The results summarized in the table seem to justify the conclusions that one of the parent cultures must have contained some albinistic hyphal elements.

Two methods could have been employed in seeking a solution of the questions involving the origin of such albinistic mycelia from ascospores obtained by mating mycelia both of which apparently produce conidia abundantly. First, a single conidium of each strain could be germinated and the two resulting mycelia mated in culture. If some albinistic ascospores were developed, the mutation responsible might have occurred before fertilization, or there might have been some such change in the sporophytic ascogenous hyphae. Although the two cases are not exactly parallel it may be pointed out that the homothallic mycelium of *Neurospora tetrasperma* produces three kinds of conidia as regards their sex alone (**5**). This suggested the advisability of comparing mycelia obtained by growing several different conidia from the same culture. The Arl.10 strain which, as noted previously is rather pale in color, appeared to be the most promising for this experiment. The Arl.6 strain would pass generally as typical for *Monilia sitophila*.

SINGLE CONIDIUM CULTURES OF ARL.10

A small tuft of aerial hyphae from a culture of Arl.10 bearing conidia was shaken in a tube of sterile water. Loops of the suspension were spread out thinly on the surface of agar in plates. After the conidia had germinated tip ends of their germ tubes were cut off and transferred. Fifteen single conidium tube cultures were obtained in this way. In all of this work no distinction is made between a conidium and the cells of conidiophore branches which disjoin and function as conidia. Within a few days it was clear that the cultures were not all alike. Ten of the cultures, numbered Arl.10.1 to Arl.10.10 would be classed as albinistic although some of them produced a very few conidia. Five cultures were producing conidia in abundance. They were not at all albinistic. One of these cultures, Arl.10.11, was chosen for further work. Nine single conidium cultures were obtained from this culture, and only one culture was albinistic. Culture Arl.10.11.a which produced conidia normally furnished conidia for a third generation of single conidium cultures. Twenty-eight of these single conidium mycelia were grown on potato dextrose agar and 7 on corn meal agar. Nineteen proved to be albinistic and 16 produced great quantities of conidia. So much aerial growth develops on potato dextrose that it is not possible to determine accurately whether some few conidia are not being formed in a culture which has all the appearance of being albinistic. Again selecting one of the cultures, Arl.10.11.a.1, which was producing conidia abundantly, 22 single conidium mycelia of the fourth generation were secured. Not a single albinistic mycelium was produced. In one case the conidium was allowed to develop further in a plate culture. Tip ends of hyphae were cut off in five different places and transferred to individual tubes. All produced conidia. Mycelium Arl.10.11.a.1 appears to be producing typical orange to golden colored conidia. The results were further checked up beginning with another culture, S, of Arl.10. Twenty-four single conidium mycelia were grown on corn meal agar and 12 on potato dextrose. Eleven of those on corn meal agar produced conidia and 13 were albinistic. Only three of the 12 on potato dextrose produced conidia. Forty-two second generation

mycelia were obtained from culture Arl.10.S.1. Of these 30 produced conidia and 12 were albinistic. Forty-five single conidium cultures from Arl.10.S.1.32 all produced conidia.

While the experiments outlined above involve only comparatively small numbers, it is evident that strainArl. 10 is unstable in that it is constantly forming some conidia the mycelia from which lack the capacity to produce conidia normally in turn. In the two series representing succeeding generations where selections of conidia for the next sowing were made from cultures producing conidia abundantly, it may be that lines were accidentally secured which are stable. Roberts (**11**) was forced to proceed to the 57th generation before he succeeded in obtaining a stable race of *Alternaria Mali* from an unstable mutant race. Since some conidia from culture Arl. 10 produce albinistic mycelia there can be no question that some sort of somatic segregation or saltation can occur after a conidium is cut off. Consider then the combination culture Arl.6 + Arl.10, which must now contain many mycelia of both strains because the culture will have been started by inoculating it with many conidia of both strains. Some Arl.10 conidia transferred furnish albinistic mycelia which can mate with an Arl.6 mycelium giving rise to hybrid asci like nos. 56 and 61. Other conidia from Arl.10 are normal and give rise to conidial mycelia which can also mate with Arl.6. All ascospores from such a mating will naturally produce mycelia which will give rise to conidia like ascus no. 58. Only in case Arl.6 should also be throwing albinistic races will there be possible a mating between two albinistic mycelia in this culture.

No ascus in which all 8 spores gave albinistic mycelia has as yet been obtained from a mating of Arl.10 + Arl.6. The one test which was made with conidia from strain Arl.6 suggests that the strain is probably a typical conidial strain which is rather stable. Conidia were sowed on agar and 28 single conidium mycelia obtained in the usual way were grown in tube cultures. After having been incubated for only 24 hours it was found that conidia were being formed in 26 of the cultures. The other two cultures showed many conidia the next day. As noted previously, some of the albinistic mycelia arising

from conidia of Arl.10 do tend to produce a few rather abnormal conidia. This is apt to occur where an aerial hypha grows down from the drying upper edge of the agar slant. The fact that some conidia are formed, even though they are not colored and are not exactly normal otherwise, is proof that the mycelium producing them possesses some conidial inheritance. It has also been found that not all of the conidial strains of the first generation sowing are equally prolific in this respect.

The size of the monilioid conidia of species of *Neurospora* is determined, in part at least, by the number of nuclei which they receive. Should some irregularity occasionally occur in the nuclear divisions preceding spore formation in strain Arl.10, one can readily see how different kinds of conidia might arise. Some conidia would be perfectly normal, others might be albinistic, and still others would be intermediates, because they would have been provided with different kinds of nuclei.

One experiment was tried out to see what would result when one of the albinistic types of mycelia derived by selection of conidia from Arl.10 was mated with an albinistic mycelium of the opposite sex derived from a hybrid ascus. Strain Arl.10.3 seems to be producing no conidia on corn meal agar. This strain was mated with haplont no. 56.6. Perithecia matured very quickly. Twenty-seven single ascospore cultures were obtained in the usual way. All of their mycelia were albinistic, indicating that such a mycelium arising through gametophytic somatic segregation does not differ so materially from one derived from an ascospore following segregation during reduction divisions of a diploid mother cell. Somatic changes described above certainly do not always result in the production of absolutely sterile albinistic races. The same thing very likely will be found to be true for segregations during meiosis. In any event an albinistic strain can be mated with a conidial strain with the result that four of the eight spores from an ascus will give rise to albinistic mycelia. Furthermore these albinistic haplonts are not all of one sex so that two of opposite sex can be mated and their progeny will all be albinistic. No other case has been reported in ascomycetes where a form derived through a mutation or saltation has been bred or reproduced

sexually to determine whether the mutation had involved factors which would be redistributed during the divisions of the nucleus of the mother cell. Such an experiment must have been possible, particularly with species of *Aspergillus*, *Neocosmopora*, *Glomerella*, *Diaporthe*, and *Penicillium*.

MICROCONIDIA

Certain ascomycetes produce two very different kinds of asexual fruit bodies. *Pezizella Lythri*[1] is a good example. The asexual Hainesia form represents the conidial stage. The patellate sporodochium is a very delicate fleshy structure which forms quantities of spores, quickly dispersed whenever supplied with moisture. The non-ostiolate pycnidium is a very resistant thick walled structure resembling a sclerotium, hence its name Sclerotiopsis. This stage is well adapted for carrying the fungus over winter. The spores of these two very unlike fruit bodies cannot be distinguished, and they serve the same purpose once they are discharged naturally. It is the structures producing the spores which differ in their secondary function. *Phoma uvicola*, the pycnidial stage of a species of Guignardia, has rather large oval to spherical spores. Along with these pycnidia, or independent of them in culture or on the host plant, there may be formed "micropycnidia," spermogonia, as they are often called, because of the many small rod-shaped spermatium-like bodies which they produce. No one has ever germinated these little bodies and opinion is divided as to their probable nature. Perithecia of species of *Guignardia* develop rather sporadically and this suggests that the "spermatia" may function in some way in bringing about fertilization. One occasionally finds a few normal pycnospores among hundreds of spermatia in spermogonia of *Phyllostictina carpogena* (l.c.). This would indicate that cells of the central region have the capacity to develop either microspores or pycnospores.

A pycnidium of *Sphaeropsis malorum* occasionally discharges small elliptical hyaline spores along with the large brown spores which are characteristic of the species. In this case the microspores do not resemble spermatia. They are probably true

[1] Jour. Agr. Res. **23**: 743–759. 1923.

spores. Pycnidia of some species of the old form genus *Phomopsis* develop two kinds of spores. Are the so-called stylospores spermatia, or are they simply paraphyses or perhaps a second kind of pycnospore?

Everyone who has cultured species of *Sclerotinia* is familiar with the minute bodies which are budded off from clusters of short flask-shaped hyphal branches. These spermatium-like bodies have long been referred to as microconidia. Once a culture has begun to produce microconidia great masses of them accumulate at the expense of the production of normal conidia. There has been much speculation as to their probable function. Brefeld (**1**), Woronin (**16, 17**), Roberts and Dunegan (**12**), Wormald (**15**) and others have tried repeatedly to germinate microconidia and have always failed. Since they do not germinate under ordinary conditions they would not seem to be a very efficient means of propagating the fungus. Humphrey (**10**) claims that the microconidia in his cultures of *Sclerotinia fructigena* germinated readily and the mycelia from them produced *Monilia* conidia. Other authors who have worked with this species are disposed to question Humphrey's statements in this regard.

It would be a difficult matter to separate the species of *Neurospora* on the basis of the shape and size of their *Monilia* conidia because there is no criterion by which to distinguish with certainty, especially in old cultures, between true conidia and fragments of conidiophores. There is also the greatest variation in the size of the true conidia. In old plate cultures one readily finds where some conidia have germinated giving rise to narrow germ tubes from the ends of which very small ellipsoid secondary conidia are budded off. These little conidia in turn germinate with still shorter and narrower germ tubes from which tertiary conidia are formed. The conidium-like supporting cells at the junction of two conidial chains are larger than true conidia, but when they become disjoined and set free they tend to round out and resemble over-sized conidia. The method of branching of the aerial hyphae of albinistic strains, as noted previously, is suggestive of a relationship of such races to a *Botrytis* or a *Monilia*, and there is a tendency to form torulose or *Polythrincium*-like

constrictions along the end branches. If they should extend to the center, a chain of true conidia would be formed in normal fashion.

Ordinarily the mycelium of *Monilia sitophila* produces conidia in great quantities and no one has even raised the question of the production of microconidia by this species. It was, therefore, of interest to learn that true microconidia were being developed in some Petri dish cultures of the albinistic races such as no. 56.6. The microspores are borne laterally and terminally on short rather blunt branches which are developed in loose clusters at intervals along a hypha, just as figured by Woronin (**17**, PL. 3, FIG. 38–44) for *Sclerotinia cinerea*. The branches are, however, from three to six cells in length and not flask-shaped (Pl. 9. *b*). The microconidia are minute hyaline spherical to pear-shaped bodies about 2.5–3.5 μ in diameter (PL. 9, *a*). Under ordinary high power one sees the bright spot at the center of the spores just as noted and figured by several authors. Examined with an oil immersion lens, however, no such spot is evident. They are at first held together in drops of water of guttation, small drops seemingly budding off from larger drops. Microspores appear to be developed usually at only one point from each cell. A sort of collar-like projection develops at that point and in optical section, it looks as though there were an opening at this point through which partly formed spores are being extruded. When first discovered the microconidia were mistaken for *Coccus* bacteria. In order to find out just what the culture appeared to be contaminated with, masses of the little bodies were spread over the surface of an agar plate in a film of water. A poured plate was also prepared in the ordinary way. Instead of developing colonies of bacteria as was expected it was found at the end of two days that short germ tubes were being formed, proving that the little bodies first taken for bacteria were really fungus spores. Microconidia swell greatly and elongate as a whole during germination (PL. 9, *e*). Tip ends of single germ tubes were cut off and transferred to tube cultures and single germinating microconidia were also isolated and transferred. The mycelium developed very slowly at first as compared with the rate of growth proceeding from a

Monilia conidium. Within a few days the cultures developed aerial hyphae and began to resemble cultures of the parent albinistic race, and it was not long before they could not be distinguished.

The mycelia derived from microconidia formed on mycelium 56.6 have been mated with the albinistic mycelium 56.2, and with mycelia derived from monilioid conidia, with the result in all cases that perithecia were formed in the usual way. This proves conclusively that the microconidia of these strains of *Monilia sitophila* are true spores. They differ from the monilioid conidia in their shape, size, manner of development and in their type of germination. They are entirely distinct morphological structures and probably are represented by a separate set of genetic factors. If so it provides another character which may be considered in breeding these fungi. Some microconidia are very likely produced in cultures of races characterized by their abundance of monilioid conidia. The secondary and teritary conidia found in plate cultures particularly, have quite a different origin, as noted above, and vary greatly in size. Microconidia have been found in plate cultures of albinistic haplonts of both sexes so that one can scarcely attribute to them the sole function being accorded to true spermatia. They are clearly homologous to the microconidia of *Sclerotinia*.

Due to their very small size, delay in germination and slowness of growth of their young mycelia, microconidia, should they be present in *Monilia* cultures like Arl.10 must always be reckoned with as a source of contamination. The albinistic saltants obtained from single monilioid conidia of Arl.10 reported previously (p. 26) can be distinguished readily on the second day. Microconidia, if accidentally transferred, would have just begun to develop branched germ tubes by this time. This work was done before it was discovered that microconidia were being developed on albinistic mycelia. As a further check, therefore, a new set of experiments was carried out. Conidia from Arl.10 were sowed on an agar plate and allowed to develop long enough to produce fresh conidia for a new sowing. The plate was carefully examined for signs of microconidia, and none being found, another sowing was made to get still another new crop

of conidia for the final test. Three days later 28 mycelia were isolated from germinating conidia. At the end of the second day it was clear that 12 cultures were producing conidia abundantly and 16 had the appearance of being albinistic. There is no question that the saltant mycelia arise directly from germinating monilioid conidia and not from microconidia.

When *Neurospora sitophila* is crossed with *N. tetrasperma* (**4**) the F_1 ascus usually develops eight spores. In the case of such interspecific hybrids it is scarcely to be expected that the f_1 haplonts would prove to be exactly like either parent in the matter of inheritance affecting the number of spores in an ascus. Sixty f_1 spores were germinated and their mycelia were tested out by mating those of opposite sex, and by back-crossing them with each of the parent mycelia. None of these f_1 haplonts was found which would, when mated, produce perithecia with either eight spored asci like the *N. sitophila* parent or four spored asci like the *N. tetrasperma* parent. From the standpoint of morphology alone one might well believe that 8-sporedness as contrasted with 4-sporedness would not be found to be determined by a simple set of conditions. The final disposition of the eight nuclei in the ascus of *N. tetrasperma* so that each spore will include a nucleus of each sex is determined largely by the orientation of the nuclear spindles. This in turn is affected by the shape of the ascus. The shape of the ascus must depend somewhat on the size of the perithecial cavity and the number of asci maturing at one time. Of the three species of *Neurospora*, *N. crassa* has the largest perithecium and the longest and narrowest asci.

In making the selections (l.c., p. 9) to obtain 8-spored and 4-spored segregates, mycelia were chosen for the resemblance of their conidial masses to those of *N. sitophila* or *N. tetrasperma*. The results of culture experiments with strains Arl.6 and Arl.10 described above indicate that production of conidia probably is entirely independent of the number of spores developed in the ascus, therefore such a method of selection was not particularly advantageous.

Some new interspecific hybrids will be described in another paper to appear shortly. At that time there will be an opportunity for a further discussion of the various questions raised

in the present paper, as well as the subject of gametophytic inheritance in general.

SUMMARY

Asci from cultures in which strains Arl.6 and Arl.10 of *Neurospora sitophila* are mated are not all alike. Some asci produce spores which differ primarily only as to their sex. All 8 spores in the ascus develop mycelia which form monilioid conidia normally. Other asci produce four different kinds of spores, two of each kind. The spores differ not only as to sex but in the types of mycelia which they develop on germination. Two spores of each sex give rise to albinistic mycelia which are practically sterile as regards their ability to produce monilioid conidia. Two spores of each sex are typical and their mycelia develop masses of orange colored conidia. In such asci the spores may alternate two and two as to sex and four and four as to being albinistic as contrasted with typical; or the spores may alternate four and four as to sex, and two and two albinistic and typical. The particular distribution of the spores in the ascus depends upon whether the factors for sex and factors for conidia are segregated during the first or the second nuclear division in the ascus.

A new race of *Neurospora sitophila* which can be propagated indefinitely by sexual reproduction has been obtained by mating two albinistic haplonts of opposite sex.

A culture of strain Arl.10, originally derived from a single ascospore, now develops two kinds of monilioid conidia. Conidia of one kind produce typical mycelia which form conidia normally; other conidia, as the result of a saltation or some sort of somatic segregation develop albinistic mycelia, which are very much like the albinistic mycelia derived from ascospores following segregations during meiosis. Some of the former, however, do produce a few colorless and otherwise abnormal conidia. By selecting conidia from Arl.10 through a few generations mycelia were obtained which appear to be typical and stable.

Strain Arl.6 is a typical stable conidial strain.

The albinistic races of both sexes develop true microconidia which are comparable to microconidia of species of *Sclerotinia*. These microconidia germinate and produce albinistic mycelia

which can be mated. The perithecia are normal in every respect except that their ascospores will be albinistic. Microconidia of *Neurospora sitophila* are distinct morphological structures not to be confused with the monilioid conidia. Whether or not typical *Monilia* strains produce microconidia has not been determined.

THE NEW YORK BOTANICAL GARDEN

LITERATURE CITED

1. **Brefeld, O.** *Peziza tuberosa* und *P. Sclerotiorum*. Bot. Unters. Schimmelpilze **4**: 112–121. *pl. 9*. 1881.
2. **Brown, W.** Studies in *Fusarium*. IV. On the occurrence of saltations. Ann. Bot. **40**: 223–243. *pl. 9*. 1926.
3. **Dodge, B. O.** Nuclear phenomena associated with heterothallism and homothallism in the ascomycete *Neurospora*. Jour. Agr. Research **35**: 289–305. *pl. 1–3 + fig. 1–5*. 1927.
4. ——. Production of fertile hybrids in the ascomycete *Neurospora*. Jour. Agr. Research **36**: 1–14. *pl. 1–4*. 1928.
5. ——. Unisexual conidia from bisexual mycelia. Mycologia **20**: 226–234. *fig. 1*. 1928.
6. ——. The nature of giant spores and the segregation of sex factors in Neurospora. Mycologia **21**: 222–231. *fig. 1–3*. 1929.
7. ——. Segregations observed in breeding the *Monilia* bread molds. Science **70**: 222. 1929.
8. **Edgerton, C. W.** The physiology and development of some anthracnoses. Bot. Gaz. **45**: 367–408. 1908.
9. **Harder, R. A.** Zur Frage nach der Rolle von Kern und Protoplasma im Zellgeschehen und bei der Übertragung von Eigenschaften. Zeits. Bot. **19**: 337–407. 1927.
10. **Humphrey, J. E.** On *Monilia fructigena*. Bot. Gaz. **18**: 85–93. *pl. 7*. 1893.
11. **Roberts, J. W.** Morphological characters of *Alternaria Mali* Roberts. Jour. Agr. Research **27**: 699–708. 1924.
12. **Roberts, J. W. & Dunegan, J. C.** The fungus causing the brown rot of fruits in America. Jour. Agr. Research **38**: 955–960. 1924.
13. **Shear, C. L. & Dodge, B. O.** Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group. Jour. Agr. Research **34**: 1019–1042. *pl. 1–4*. 1927.
14. **Wilcox, M. S.** The sexuality and arrangement of the spores in the ascus of *Neurospora sitophila*. Mycologia **20**: 3–16. 1928.
15. **Wormald, H.** The "Brown Rot" diseases of fruit trees, with special reference to two biologic forms of *Monilia cinerea*, Bon. II. Ann. Bot. **34**: 143–171. *pl. 4, 5*. 1920.
16. **Woronin, M.** Über die Sclerotienkrankheit der Vaccinieen-Beeren. Mem. Acad. Imp. Sci. St. Petersbourg. VII.E **36**: 1–49. *pl. 1–10*. 1888.
17. ——. Über *Sclerotinia cinerea* und *Sclerotinia fructigena*. Mem. Acad. Imp. Sci. St. Petersbourg. VIII. **10**: 1900.

EXPLANATION OF PLATES

Neurospora sitophila

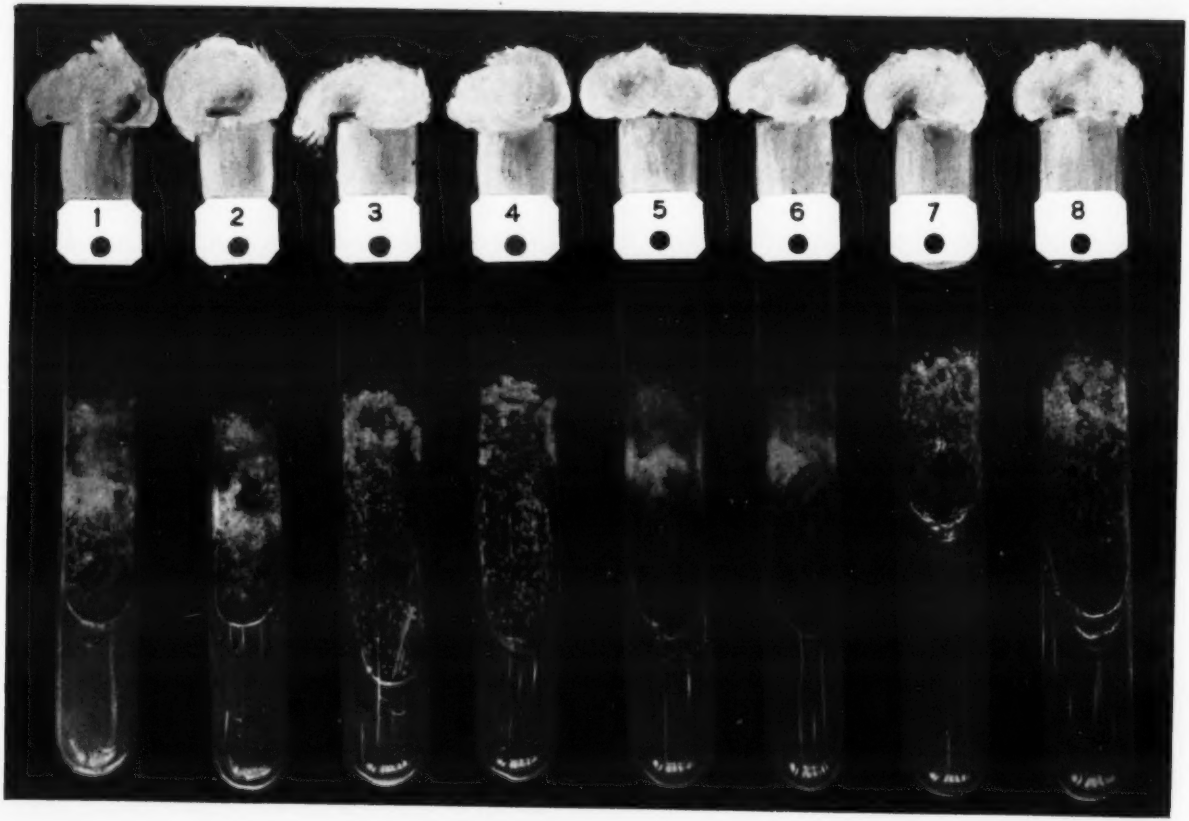
Small green circles on tubes indicate haplonts A; red circles, haplonts B.

PLATE 8

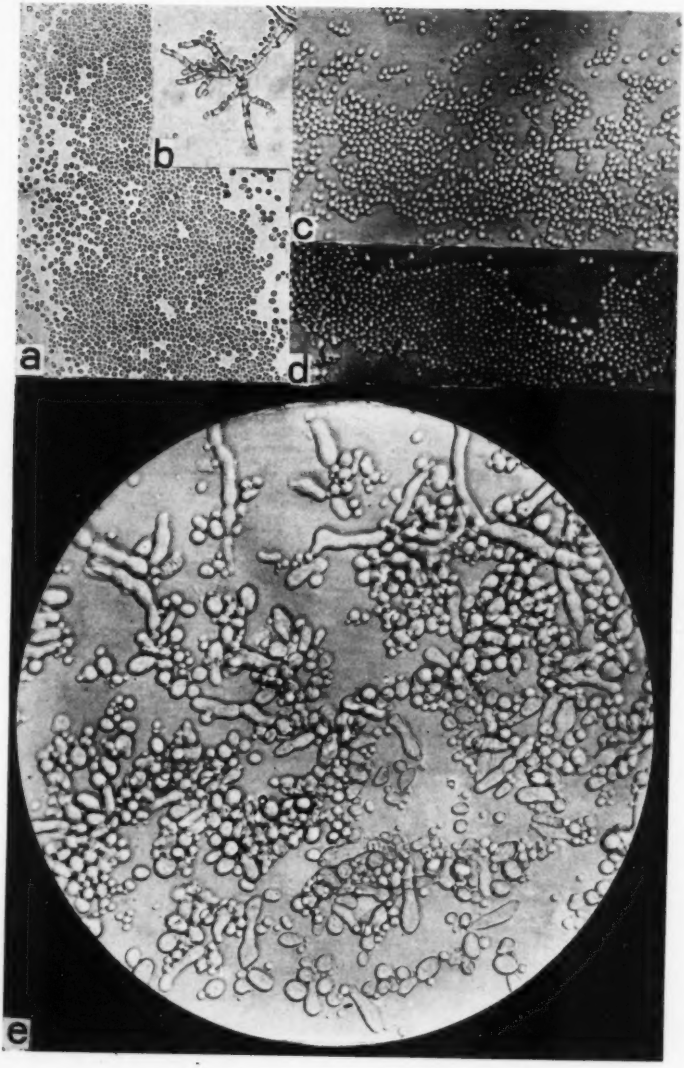
Cultures of the eight haplonts derived from ascus no. 56 from a mating of strains Arl.6+Arl.10. Albinistic haplonts nos. 1, 2 and 5, 6 are practically sterile as to production of conidia. Haplonts nos. 3, 4 and 7, 8 are normal for the species, producing an abundance of conidia. Segregation of the sex factors occurred in the first nuclear division in the parent ascus and segregation of factors for conidia in the second division. Four genotypically different kinds of clons from one mother-cell.

PLATE 9

a–d. Microconidia from a plate culture of the albinistic clon no. 56.6. b. Small branches producing microspores. e. Germinating microconidia from the same sowing about 40 hours later. Note how the microspores swell during germination. (a, b and e × 270; c, d × 315.)



MONILIA SITOPHILA



MONILIA SITOPHILA
(Microconidia)

STRAW COMPOST FOR MUSHROOM CULTURE [1]

ILLO HEIN

Artificial farmyard manure with many of the properties of composted stable manure has frequently been made with success from straw. The use of such artificial manure for mushroom growing, however, has hitherto been given little attention and is not reported in the literature. Studies on substitutes for the mushroom compost now in use are being conducted in the department of Botany at The Pennsylvania State College and for this purpose an experimental mushroom house has been constructed (Hein, 1929).

That cellulose splitting organisms require an abundant supply of available nitrogen is generally known and this fact has been studied especially by investigators interested in the conservation of nitrogen in the soil. Carbohydrates are said to provide energy food for, and thus stimulate the growth of, nitrogen feeding organisms. For the decomposition of cellulose it appears that a larger supply of nitrogen than is ordinarily found in straw, corn stover, hay, etc., is necessary. Studies on the natural decomposition of cellulose have shown that both chemical and biological activities are concerned. The data in the literature under this head has recently been summarized by Bradley and Rettger (1927). The decrease in nitrate supply due to the presence of plant food materials of high carbon content has been reported by Kellerman and Wright (1914), Hill (1915), Doryland (1916), Lemmerman and Einecke (1919), Briscoe and Harned (1918), Rahn (1919), and many others. Collison and Conn (1928) state that cellulose has a stimulating effect on nitrate feeding organisms but also find that plant products high in cellulose have a toxic effect on young seedlings.

Hutchinson and Richards (1921) have reported the production from straw of a well rotted manure which closely resembles

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as Technical Paper No. 489.

ordinary stable manure in color, structure and odor. According to them the essential conditions for making artificial manure from straw are, air supply, suitable temperature, and a supply of soluble nitrogen compounds as food for the fermentation organisms. A slightly alkaline reaction is necessary and this should never be higher than the upper limit of tolerance of the organisms. Halverson and Torgenson (1927) report similar successful studies and state that the soluble nitrate compounds applied to the straw as food for bacteria, ammonium sulphate with the addition of lime to neutralize the acids formed, proved most satisfactory and economical. Albrecht and Poirot (1928) attempted the practical application of the methods of previous workers and produced good manure from wheat straw. Field tests using straw manure showed improvement in yields of wheat and of sweet clover, the yield in the latter case being even greater than that obtained from the application of barnyard manure.

Wheat straw was composted in heaps to which nitrogen compounds in various forms were applied. The composting was carried on outdoors during the spring months and indoors during the winter months.

The following tables give the results of some of the successful tests in mushroom production from the manure made by composting straw both with and without the addition of other food materials for the cellulose decomposing organisms. The compost heaps were made up in closely adjoining piles approximately 4 x 9 x 8 feet high. The pile in the beginning was many times the volume of later stages when the straw had lost its stiffness. The volume reduction of the straw after decomposition was similar to that shown in figure 1, p. 125, by Albrecht and Poirot (1928). This fact made the straw pile a little difficult to handle. Both treated and untreated heaps showed a rapid rise in temperature after a few days. The greatest difficulty was experienced in thoroughly wetting the straw during the early stages. Since the water rapidly seeped through the comparatively loose piles, the straw could not in the beginning be adequately moistened. The method of at first watering the straw lightly as suggested by Hutchinson and Richards (1921) and then watering heavily

TESTS MADE WITH WHEAT STRAW

Nitrogen compound added to straw	Amount	pH at time of making up beds	Mycelial growth	Yields per sq. ft. during first 28 das. of bearing period
Manure from old spent mushroom bed	1 part in 10 by vol.	7	Slow at first but good growth later	8 oz.
	1 part in 50 by vol.	7.5	Good growth	5 oz.
	1 part in 100 by vol.	6.5	Good	6 oz.
Rich garden soil	1 part in 10 by vol. 1 part in 50 by vol.	7 6.5	Fair growth in beginning	0 Few buttons
	1 part in 100 by vol.	6.5	Good	6 oz.
Fresh horse manure	1 part in 10 by vol.	7	Fair	Few buttons died later
	1 part in 50 by vol.	7.5	Fair	8 oz.
	1 part in 100 by vol.	7.5	Good	4 oz.
Liquid horse manure	20 gal. per 100 lbs. straw	7	Good	12 oz.
Ammonium nitrate	1 lb. per 100 lbs. straw	7.8	Fair	5 oz.
Ammonium sulphate and lime	1 lb. per 100 lbs. straw	6.5	Very good	4 oz.
Ammonium phosphate	1 lb. per 100 lbs. straw	6.5	Very good	5 oz.
Ammonium carbonate	1 lb. per 100 lbs. straw	7	Good	0
Check			Good	13 oz.

after the initial fermentation had been going on for a few days proved satisfactory from the point of view of water absorption by the straw.

Where good garden soil or manure was used as a source of nitrate the material was simply stirred with the straw by forking over. The soluble nitrates were dissolved in water and applied

with a watering can four days after the initial fermentation had begun.

The H-ion content was determined by the colorimetric method just before the finished product was made up in beds. Yield was estimated on the basis of average weight of the mushrooms per square foot. Yields were poor as compared with those obtained in the horse manure check beds and with the yields ordinarily obtained in commercial practice. Certain patches produced no mushrooms while others produced a fair stand. The low yields were perhaps due more to unfavorable environmental factors, since with the equipment available at the time, humidity and temperature conditions could not be adequately maintained. I am of the opinion that better yields than those here recorded can be obtained with straw manure under more favorable environmental conditions and further experimental work along these lines is under way.

The results obtained show no consistency nor can conclusions be drawn other than that mushrooms can be produced on straw manure. The yield from beds of horse manure compost made up at the same time as the above beds were only slightly better so that undoubtedly other factors were responsible for the low yields. Under properly managed environmental conditions better production should be obtained.

Further studies with straw and other substitutes for horse manure are soon to be reported.

DEPARTMENT OF BOTANY,
THE PENNSYLVANIA STATE COLLEGE

LITERATURE CITED

1. **Albrecht, W. A. & Poirot, E. M.** Farm Trials of Artificial Manure. Jour. Am. Soc. Agron. **20**: 123–132. 1928.
2. **Bradley, Leon & Rettger, L. F.** Studies on aerobic bacteria commonly concerned in the decomposition of cellulose. Jr. Bact. **13**: 321–345. 1927.
3. **Briscoe, C. F. & Harned, E. H.** Bacteriological Effects of Green Manure. II. Mississippi Agr. Exp. Sta. Bull. No. 185, 3–18. 1918.
4. **Collison, R. C. & Conn, H. J.** Artificial Manure from Straw. N. Y. Agr. Exp. Sta. Circ. 95. 3 p. 1928.
5. **Doryland, C. J. T.** The influence of energy material upon the relation of soil micro-organisms to soluble plant food. North Dakota Agr. Exp. Sta. Bull. No. 116, 320–401. 1916.

6. **Halverson, W. V. & Torgenson, E. F.** Production of artificial farmyard manure by fermenting straw. Jour. Am. Soc. Agron. **19**: 577–594. 1927.
7. **Hein, Illo.** An Experimental Mushroom House. Torreya **29**: 131–132. 1929.
8. **Hill, H. H.** The effect of green manuring on soil nitrates under greenhouse conditions. Virginia Agr. Exp. Sta. Bull. No. 6, 121–153. 1915.
9. **Hutchinson, H. B. & Richards, E. H.** Artificial farmyard manure. Jour. Min. Agr. Great Britain **28**: 398–411. 1921.
10. **Kellerman, K. F. & Wright, R. G.** Relation of bacterial transformations of soil nitrogen to nutrition of citrous plants. Jour. Agr. Res., **2**: 191–113. 1914.
11. **Lemmerman, O. & Einecke, A.** Über den Stickstoffsinshalt der Boden und die Wirkung von Stron und Zucker. Landw. Ver. Sta., **93**: 209–229. 1919.
12. **Rahn, O.** Die shädliche Wirkung der Strohdungung und dessen Verhutung. Zeitschr. Tech. Biol., **7**: 172–186. 1919.

NOTES AND BRIEF ARTICLES

Mushrooms of Field and Wood is the title of a small well illustrated volume just issued by Miss Margaret McKenny and published by the John Day Company of New York. Many of the older and popular volumes on mushrooms are out of print and inaccessible and one is always interested in the new volumes that are coming off the press. The editor of MYCOLOGIA is repeatedly called upon to recommend books which can be used by the amateur who wishes a very general knowledge of the common forms to be found in the woods and fields. This volume has been issued primarily to fill such a need. While it is not intended exclusively for the mycophagist, many of the poisonous and edible species are illustrated. Most of the space is devoted to the Basidiomycetes. Only a limited space is devoted to the Ascomycetes. The work is intended largely for the use of Girl Scouts and similar organizations, and will doubtless be widely used by young nature lovers who wish to familiarize themselves with these plants.

A most important addition to botanical literature has recently been issued from the Farlow Herbarium of Cryptogamic Botany and dedicated to the memory of William Gilson Farlow. This Host Index of the Fungi of North America, published June 10, 1929, has been compiled by Professor Arthur Bliss Seymour and includes in its 732 pages some 80,000 names of hosts and fungous parasites. Bacterial pathogens of plants and fungous parasites, with a few exceptions, of the higher animals, including man, are not listed in this work. The geographical area includes the whole of North America, the Island of Trinidad and Panama to the northern limits of plant life from Greenland to Alaska. One must agree with the compiler that no pains have been spared to make the index as complete and accurate as possible. It has been attempted to include all reports in the literature covering the area mentioned for the past 50 years. Mycologists and pathologists will find this work of great value.

The Fungi of Manitoba by G. R. Bisby, A. H. R. Buller and John Dearness with a preface by E. J. Butler, Director of the Imperial Bureau of Mycology at Kew, England, has just been issued. This book includes a record of nearly 2000 species of fungi which have been collected in the province of Manitoba, 45 of which are described as new. The list is preceded by a general discussion of 49 pages and is followed by a host index and an index of substrata and also an index of classes, orders, families and genera. The list includes a number of rare species. In the Discomycetes, the writer is interested to note the record of some rare forms, among these, *Underwoodia columnaris*, *Ascobolus striisporus*, *Elvela sphaerospora* and *Plectania hiemalis*. The last-named species which is apparently common about Winnipeg has been encountered by the writer just once in the preparation of North American Cup-fungi. Since the fungi are quite cosmopolitan the list presented here would probably fit most any region in temperate North America. The volume is very neatly gotten up and a creditable piece of work. Two of the authors, A. H. R. Buller and J. Dearness are Associate Editors of MYCOLOGIA. Copies are available from Prof. V. W. Jackson, Manitoba Agricultural College, Winnipeg, Canada, at two dollars and fifteen cents, postpaid.

TWO NOTABLE PAPERS ON SLIME MOULDS

1. In the latest volume of the *Transactions of the Royal Society, Edinburgh*, the opening paper—issued indeed as a "separate," March, 1928—has perhaps only recently reached readers in the open libraries of the world.

This paper is in every way important. It makes appeal to the readers of *Mycologia* but almost quite as well to all morphologists, cytologists at least, whether students of plants or animals. The subject is nothing more nor less than the "*Life-history and Cytology* of *Reticularia Lycoperdon* Bull.," one of the larger æthalioid species of the slime moulds. From field to laboratory and from laboratory to field again, for many months, Dr. Malcolm Wilson and Miss Elsie J. Cadman, aided by the latest physical equipment and chemical reagents, have at the University of Edinburgh pursued their researches, now

before us as a beautiful monograph of nearly a hundred quarto-pages, followed by seven fine collotype plates, bearing more than a hundred figures, chiefly from the microscope; bringing to the careful reader almost a personal experience of the facts which support the evenly progressive argument of the authors.

This note is not intended to be a review; those interested will read for themselves. But readers of MYCOLOGIA will be interested to know that, in the group named, life-histories are the exception; that few species have been set out with the fullness of detail seen here; none in such luxurious form. Dr. Jahn of Münden, Hannover Germany, has worked far longer, and has examined a number of species, especially the STEMONITES; but facilities for publication and illustration have not been at his disposal. Dr. Jahn's research has covered much the same ground and his work is recognized by the Scots throughout, and—except in one notable instance—is confirmed.

The spores of the Myxos, as all students know, give rise to amœbæ which presently become ciliated swarmspores; but later—as hitherto reported—resume the amœboid condition and conjugate to form the units whose repeated division and association constitute the vegetative, sporophytic phase—the *plasmodium*. In the *Reticularia* species now before us the *swarm-cells themselves* conjugate and form the plasmodium directly, losing their cilia. The zygote *is* the plasmodium and is nourished—at the outset, at least—by coalescent non-conjugant swarmspores; myxamœbæ in *Reticularia* are none.

But as stated this does not pretend to be a review; it is but an attempt to call attention of naturalists to an important piece of work. Curiously enough, the authors find themselves not seldom in presence of situations not dissimilar to those characterizing plants—the higher plants in fact—the sequence of meiosis, notable.

2. *Les Myxomycetes de Neamtz* (Moldavie), par M. le Professor Marcel Brandza, *Extr. du Bulletin de la Soc. Mycol. de France, Tome XLIV*, Fascic. 3, pp. 249–299; pl. xiv–xvii.

Professor Brandza is a member of the staff of the University of Roumania, Bucarest. The work, of which he tells the world, was done at a distant point in the growing kingdom, viz.:—at

"Neamtz"; and to save the blushes of readers and reviewers generally, he kindly includes, parenthetically, the name of the province in which "Neamtz" has place—*Moldavie*—(en francais).

Prior to the world war, as all men know, Roumania consisted mainly of two provinces; south, Wallachia, extending east and west, from the Iron Gate to the Black Sea; a well-watered plain, between the Danube and the Transylvanian Alps; and north, Moldavia, stretching north and south, the Sereth, its river, draining the eastern face of the Carpathians, the topography a dissected peneplain, as Stieler shows it—cut and carved by river, fork, branch, brook and rivulet—back to the perennial springs that seep from mountain-sides or banks of frozen snow.

Now as history will have it, the name Neamtz is at once that of a river, a city and a *monastery*, all associated in the northwest corner of the province; the Moldovia river, if you please, of the Sereth a principal fork, receives a branch, the Neamtz water, as if direct from the mountains; but itself breaking, dividing, amid the rising foothills, into brooks and rivulets uncounted. On one of these brooks, branch of the Neamtz river, the old monastery finds its place.

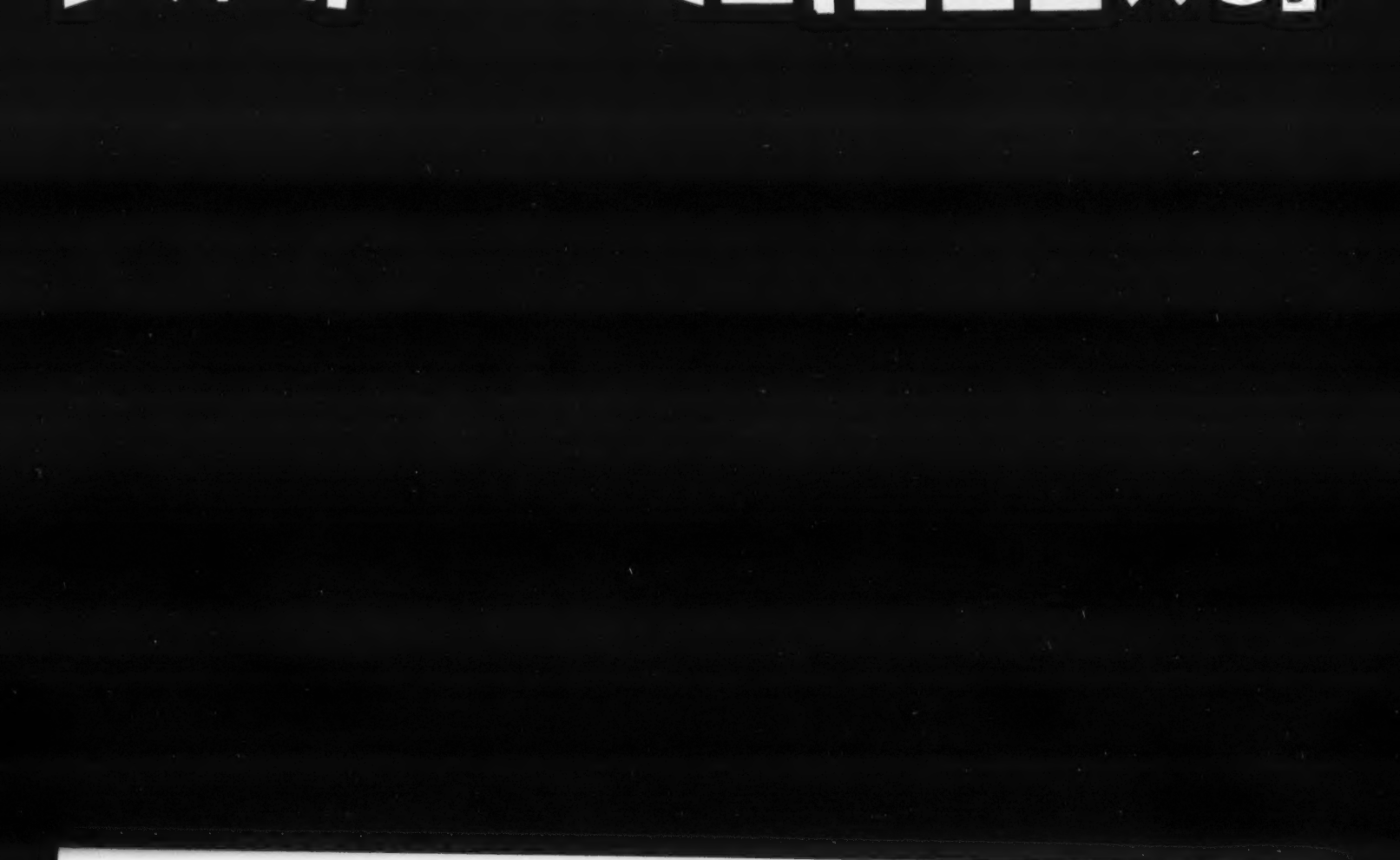
Fine! This page before him, any naturalist would say: This is a place for trees! Here we look for forest primeval; and lo—here the forest stands. Not the forest primeval perhaps, but no doubt a very considerable part of it, saved in large part by conservative monks, who in forest and high cañon found escape from destruction by Turk and Crusader, and for centuries controlled vast areas down to times quite recent. In 1864, indeed, the government resumed a large part of the really most valuable woodlands, leaving to the monasteries greatly diminished holdings, but after all, sufficient to show some of the ancient woodland quite in primitive condition—which brings us to Professor Brandza's thesis. The scholarly professor, a student of Myxomycetes, proposes for the group cosmopolitism, established by the number of species taken at and around the old monastery as centre.

He has been at work twelve years within an area not exceeding four miles in diameter, nowhere surpassing 2300 feet in highest altitude. Within this small but charmed circle, he finds 181

species, of which he gives annotated descriptions; two of these and one variety, new to science, he figures in color. He finds that he has 84 per cent of the forms of Europe, considering the altitude limitation, and 76 per cent of European species as a whole.

Besides this he has found many species, hitherto discoverable only in other parts of the world; or, if in Europe at all, only rarely in this list we find several of our American forms.

Citing all these remarkable facts our author concludes: "The concentration of so great a number of species upon a surface so restricted as that of our researches at Neamtz, affords certain proof that the conditions favorable to the life of these lower organisms (humidity, substratum, warmth) are the only factors essential, determining their appearance in any region anywhere upon the globe. If we except the Alpine species we may say that all other species show in general a manifest tendency to cosmopolitism."

That we may see the extent and real character of his small domain we are early confronted by a little map in the text, which illustrates beautifully the erosional topography—the hills and valleys and streams which an abiding fraction of the deciduous forest about the monastery of Neamtz no doubt still overshadows and adorns.

T. H. MACBRIDE